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Im Auftrag

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Novel method for the production of anti-human antigen receptors and uses thereof

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Novel method for the production of anti-human antigen receptors and uses thereof

The present invention relates to a method for the production of an anti-human antigen receptor that is low or not immunogenic in humans comprising the steps of selecting a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least said VH chain is derived from essentially unprimed mature human B-lymphocytes or from essentially anergic human B cells and said VL chain is derived from a naturally occurring human B cell repertoire, said chains being expressed from a recombinant vector and using an in vitro display system for binding to a human antigen.

The present invention further relates to receptors that are low or not immunogenic in humans and directed to human antigens, said receptors being obtainable by the method of the invention. Said receptors are preferably antibodies or fragments thereof or immunoconjugates comprising the VH/VL chains of said antibody. In particular, the receptors of the invention are directed to human tumor antigens, preferably to the human tumor antigen 17-1A, also known as EpCAM, EGP or GA 733-2. Finally, the present invention relates to kits useful for carrying out the method of the invention.

The mammalian immune systems such as the human immune system select against immune competent cells and molecules that are specific for self-antigens. Dysregulation of the immune system in this regard may result in auto-immune diseases such as rheumatoid arthritis. In general, the surveillance of the immune system with regard to the autoreactive antibodies or T cells is therefore highly beneficial. However, there may be cases where it would be advantageous to have autoreactive antibodies that are directed to antigens expressed in the mammalian, and in particular, the human body. Such antigens are, for example, tumor associated antigens. For example, the human 17-1A or EpCAM antigen, a surface glycoprotein expressed by cells of simple epithelia and malignant tumors derived thereof, has been shown to be a rewarding target for monoclonal antibody therapy of cancer,

especially in patients with minimal residual disease suffering from disseminated tumor cells that may cause later solid metastasis and thus impairs patients' prognosis. In patients with minimal residual colorectal cancer, a murine monoclonal antibody specific for the human 17-1A-antigen decreased the 5-year mortality rate by 30% compared to untreated patients, when applied systemically in five doses within four months after surgery of the primary tumor; in total each patient was treated with 900 mg of antibody (Riethmüller, Lancet 343 (1994), 1177-1183). However, during the course of antibody treatment patients developed a strong antibody response against the murine immunoglobulin. These human anti-mouse antibodies (HAMAs) severely limit the continuous application of antibody therapies and increasingly reduce their efficacy. Moreover, preformed HAMAs induced by former antibody treatment or another contact with murine immunoglobulin can severely interfere with later antibody therapies.

To prevent these problems, therapeutic antibodies with minimal immunogenicity would be preferable. To achieve this goal, it might be, for example, envisaged that therapeutic antibodies or antibody derivatives are completely human by their amino acid sequence and the immunogenic profile of the human antibody idiotype is minimized by using human Ig-variable regions likely to be tolerated by the human immune system.

However, the generation of human antibodies against human antigens faces two major problems:

(1) Hybridoma or other cell immortalisation techniques proved to be quite inefficient in generating human antibody producing cell lines compared to the murine hybridoma technology.

(2) Auto-reactive antibodies are relatively efficiently depleted of naturally occurring antibody repertoires due to the mechanisms mediating self-tolerance.

Human antibodies in general have become much more accessible since the availability of transgenic mice expressing human antibodies (Brüggemann, Immunol. Today 17 (1996), 391-397) and of the combinatorial antibody library and phage display technology allowing the in vitro combination of variable regions of Ig-heavy and light chains (VH and VL) and the in vitro selection of their antigen binding specificity (Winter, Annu. Rev. Immunol. 12 (1994), 433-455). By using the phage display method, rare events like one specific binding entity out of 10^7 to 10^9 different

VL/VH- or VH/VL-pairs can easily be isolated; this is especially true when the repertoire of variable regions has been enriched for specific binding entities by using B-lymphocytes from immunized hosts as a source for repertoire cloning.

Often, however, the frequency of specific binding entities is substantially lowered in naturally occuring antibody repertoires. This is particularly true for cases of antibodies binding to self-antigens. Random combinations of VL- and VH-regions from a self-tolerant host resulting in a combinatorial antibody library of conventional size (10^7 to 10^9 independent clones) most often are not sufficient for the successful in vitro selection of auto-reactive antibodies by the phage display method.

One strategy to circumvent this problem is the use of very large combinatorial antibody libraries that compensate by the library size for the low frequency of auto-reactive antibodies in naturally occuring repertoires. Combinatorial antibody libraries exceeding a size of 10^9 independent clones, however, are difficult to obtain because of the current technical limit of the transformation efficiency for plasmid-DNA into E.coli-cells.

To avoid the self-tolerance mediated bias in naturally occuring antibody repertoires, that underrepresents auto-reactive antibodies and markedly decreases the chances of isolating antibodies specifically recognizing self-antigens, approaches using semisynthetic or fully synthetic VH- and/or VL-chain repertoires have been developed. For example, almost the complete repertoire of unrearranged human V-gene-segments has been cloned from genomic DNA and used for in vitro recombination of functional variable region genes, resembling V-J- or V-D-J-recombination in vivo (Hoogenboom, J. Mol. Biol. 227 (1992), 381-388; Nissim, EMBO J. 13 (1994) 692-698; Griffiths, EMBO J. 13 (1994), 3245-3260). Usually, the V-D-/D-J-junctional and the D-segment diversity mainly responsible for the extraordinary length and sequence variability of heavy chain CDR3 as well as the V-J-junctional diversity contributing to the sequence variability of light chain CDR3 is imitated by random sequences using degenerated oligonucleotides in fully synthetic and semisynthetic approaches (Hoogenboom (1994), supra; Nissim, supra; Griffiths, supra; Barbas, Proc. Natl. Acad. Sci. U.S.A. 89 (1992), 4457-4461).

However, VL/VH- or VH/VL-pairs selected for binding to a human antigen from such semisynthetic or fully synthetic repertoires based on human V-gene sequences are at risk of forming immunogenic epitopes that may induce an undesired immune

response in humans (Hoogenboom, TIBTECH 15 (1997), 62-70); especially the CDR3-regions derived from completely randomized sequence repertoires are predestined to form potentially immunogenic epitopes as they have never had to stand the human immune surveillance without being recognized as a foreign antigen resulting in subsequent elimination. This is equally true for human antibodies from transgenic mice expressing human antibodies as these immunoglobulin molecules have been selected for being tolerated by the murine but not the human immune system.

Accordingly, the technical problem underlying the present invention was to provide a method that allows the production of receptors that are low or not immunogenic in humans and that can be used for targeting antigens in the human body. The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Thus, the present invention relates to a method for the production of an anti-human antigen receptor that is low or not immunogenic in humans comprising the steps of selecting a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least said VH chain is derived from essentially unprimed mature human B-lymphocytes or from essentially anergic human B cells and said VL chain is derived from a naturally occurring B cell repertoire, said chains being expressed from a recombinant vector and using an in vitro display system for binding to a human antigen.

The method of the present invention is highly advantageous for providing receptors that can be used for targeting antigens in humans without being at all or to any significant extent immunogenic themselves. Such receptors can advantageously be used for treating a variety of diseases such as tumors or auto-immune diseases, graft rejection after transplantation, infectious diseases by targeting cellular receptors as well as allergic, inflammatory, endocrine and degenerative diseases by targeting key molecules involved in the pathological process.

The VH/VL immunoglobulin chains of the receptors of the present invention can, of course, be further investigated with regard to their nucleic acid and amino acid sequences using techniques well-known in the art, see e.g. Sambrook (Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbor, NY (1989)). Once the nucleic acid sequence or the amino acid sequence have been determined, the receptors of the invention can also be produced by other methods, such as by synthetic or semi-synthetic methods yielding synthetic or semi-synthetic receptors, or in transgenic mice expressing human immunoglobulin receptors; carrying the features recited herein above and produced by such synthetic or semi-synthetic methods or in said transgenic mice are also included within the scope of the present invention.

After binding of the receptor to the human antigen, the receptor can be further purified. For example, non-bound receptors which do not carry the antigen specificity may be removed by washing steps. The bound receptor may be eluted from the human antigen and further purified, wherein additional rounds of expression, binding and selection of the desired receptor may be used until the desired receptor and/or the corresponding recombinant vector have been isolated in pure form.

The method of the present invention thus makes use of the preselection of Ig-variable regions by the human immune system. The receptors are derived from a repertoire selected in vitro from human combinatorial antibody libraries exclusively or preferentially made of the naturally occurring antibody repertoire expressed by essentially unprimed mature human B-lymphocytes or from essentially anergic human B-cells.

However, the Ig variable domains may also be derived from a variety of other sources that represent these preselected populations of B cells.

The scientific background with regard to the origin of the B cells functioning as a source of said VH or VL chains, may be explained as follows:
Mature unprimed B-lymphocytes, expressing IgD and IgM as membrane antigen receptors enter primary follicles during their traffic to and between secondary

lymphoid organs unless they have encountered multivalent self antigen resulting in clonal deletion or soluble monovalent self antigen rendering them anergic and short-lived due to exclusion from primary follicles.

Contact of immature B-cells, that exclusively express IgM, with self antigen in the bone marrow results in clonal deletion or anergy depending on the antigen valency. Anergic B-cells, although expressing surface IgD, are unable to respond to the antigen through this receptor; without access to primary follicles and in the absence of T-cell help, these cells have a short half-life of only a few days.

In contrast, mature unprimed B-lymphocytes that have not encounted self antigen and therefore have access to primary follicles have a long half-life of several weeks. Despite the described mechanisms mediating self-tolerance, these populations of long-lived mature unprimed B-lymphocytes still contain potentially self-reactive B-cells, that are, however, unlikely to find specific T-cell help due to T-cell tolerance and thus kept from proliferation and antibody secretion. It appears that B-cell non-responsiveness to many self-antigens that are present at low levels is of this type, affecting the helper T-cells but not the B-cells. In the present invention these long-lived, non-responsive, potentially self-reactive mature unprimed B-lymphocytes have been identified as the most promising naturally occuring human antibody repertoire for constructing combinatorial antibody libraries especially suited to select human antibodies to human antigens by, for example, the phage display method.

This highly selected antibody repertoire used as a basis for the present invention mainly derived from B-cells with a long in vivo half-life and thus exposed to the human immune system for prolonged periods of time is markedly depleted of antibody molecules forming epitopes especially within the highly variable CDR3-regions, that are immunogenic for the human immune system. Therefore, human antibodies selected from this antibody repertoire are expected to have a lower immunogenic profile in humans than human antibodies selected from semisynthetic or fully synthetic human antibody libraries.

Mature unprimed B-cells that are activated by contact with foreign antigen stop to express IgD and start clonal proliferation and differentiation into plasma cells secreting soluble immunoglobulin; early stages of the antibody response are dominated by IgM-antibodies, while later, IgG and IgA are the predominant isotypes, with IgE contributing a small but biologically important part of the antibody response.

Unlike IgD-negative mature antigen-primed B-lymphocytes expressing IgM, IgG, IgA or IgE, IgD-positive mature unprimed B-cells have not yet undergone clonal proliferation, so that combinatorial IgD-libraries do not overrepresent antibody specificities that are currently or have been formerly involved in immune responses usually driven by foreign antigen, thus decreasing repertoire diversity and wasting library space for antibody candidates unlikely to bind self antigen. This is in clear contrast to the prior art recommending the use of human IgM combinatorial antibody libraries for the in vitro selection of human antibodies against human antigens from naturally occuring human antibody repertoires (Hoogenboom (1997), supra).

In a preferred embodiment of the method of the invention, said antigen receptor is an immunoglobulin or a fragment thereof.

The fragment of the immunoglobulin may be any fragment that is conventionally known in the art such as Fab or $F(ab)_2$ fragments.

In a particularly preferred embodiment of the method of the invention, said immunoglobulin fragment is an Fv-fragment.

In a further preferred embodiment of the method of the invention, at least said VH and optionally said VL immunoglobulin chain are derived from a human IgD repertoire.

This receptor and preferably antibody repertoire selected for low immunogenicity has been concluded to be best represented in a human IgD-antibody library. IgD is expressed as membrane antigen receptor together with surface IgM on mature unprimed B-lymphocytes that enter primary follicles during their traffic to and between secondary lymphoid organs unless they have encountered multivalent self antigen resulting in clonal deletion or soluble monovalent self antigen rendering them anergic and short lived due to exclusion form primary follicles. Except mature unprimed B-lymphocytes human IgD-libraries only represent the antibody repertoire of short-lived B-cells that have been rendered anergic in contact with soluble monovalent self antigen but are unlikely to contribute specific binders to human cell

surface molecules resembling multivalent self-antigens that induce clonal deletion instead of B-cell anergy.

In a further preferred embodiment of the method of the invention, said in vitro display system is a phage display system.

The phage display system has, in the past, conveniently been used for the selection of a variety of peptides and proteins that bind to specific targets. On the basis of this knowledge, the immunoglobulin VH and VL chains can conveniently be cloned into vectors that also comprise molecules useful for phage display systems. Such molecules and vectors, respectively, are well-known in the art (Winter, supra; Barbas, METHODS: A Companion to Methods in Enzymology 2 (1991), 119-124) and need not be explained here in more detail.

In a further preferred embodiment of the method of the invention, said combination of rearranged chains is expressed from one or more different libraries.

This embodiment is particularly preferred, if a VH or VL chain is known that binds to a specific target and the corresponding second V chain that reconstitutes or improves binding is selected.

In a further preferred embodiment of the method of the invention, said human antigen is a tumor antigen.

If the human antigen is a tumor antigen, said antigen is preferably expressed on the surface of said tumor. In this case, the VH and VL chains are advantageously coupled to a toxine. The coupling can be effected on the nucleic acid level by genetic engineering or at the protein level by, for example, chemical coupling.

It is particularly preferred that said tumor antigen is the 17-1A antigen.

In a further particularly preferred embodiment of the method of the invention, said VH chain comprises one of the two sequences shown in Fig. 6 (nucleotides 1 to 381)

and Fig. 7 (nucleotides 1 to 339) and/or said VL chain comprises the sequence shown in Fig. 5 (nucleotides 1 to 321). Receptors with these specific VH and VL regions, wherein said VL region can be combined with both VH regions, are the first human antibodies that are specific for the human 17-1A antigen.

In a further preferred embodiment of the method of the invention, said selection step involves (i) binding of the display vehicle expressing an antigen receptor

(a) on immobilized target antigen or fragments thereof

(b) on optionally labeled cells expressing the target antigen or fragments thereof

(c) or to soluble, preferably labeled target antigen or fragments thereof;

(ii) washing off non-specifically binding display vehicle (a and b) and subsequent elution of specifically binding display vehicle by non-specific (e.g. low pH buffer) or specific means (e.g. target antigen specific antibody) or (iii) positive enrichment of target antigen bound display vehicle (b and c) from target antigen solution or from suspensions of cells expressing the target antigen for example using magnetic beads binding to labeled target antigen or labeled cells expressing the target antigen respectively; thus isolated display vehicles including their antigen receptors optionally being multiplied by replication and subjected to further rounds of in vitro selection as described.

In a further preferred embodiment of the method of the invention, prior to said selection step either said VH or said VL chain is selected for binding to said antigen together with a surrogate V chain.

This two-step procedure can be employed using a target antigen specific template antibody from a different species, for example a murine monoclonal antibody against the human target antigen. First, a human VL- or VH-repertoire is combined with a single surrogate VH- or VL-chain from the murine template antibody, displayed, e.g., on filamentous phage and selected in vitro for antigen binding. Thus the complete library size is available exclusively for the human VL- or VH-repertoire and candidate human VL- or VH-chains can be isolated that are capable of contributing to specific binding of the human target antigen. In a second step, the surrogate variable region of the template antibody is replaced by the corresponding human variable region

repertoire followed by a second round of in vitro selection; again, the complete library size is exclusively available for a single VH- or VL-region repertoire, thus enabling much more VL- and VH-region candidates to be screened for antigen binding under conditions of limited library size by the two-step procedure than by a single-step procedure.

For cloning of DNA-sequences encoding the variable regions of human antibodies that specifically bind to the human 17-1A-antigen, this two-step selection procedure for screening human IgD-combinatorial antibody libraries by the phage display method was advantageously employed. First, the Fd-heavy chain segment (VH+CH1) of the murine monoclonal antibody M79 (Göttlinger, Int. J. Cancer 38 (1986), 47-53) that specifically binds to the human 17-1A-antigen was combined with a human kappa- and lambda-light chain repertoire respectively. The resulting libraries were displayed on filamentous phage and selected in vitro by several rounds of panning on immobilized recombinant human 17-1A-antigen. Soluble Fab-fragments were expressed from several clones after each round of panning and screened by ELISA for antigen binding. Each of the strong binding entities enriched during the panning procedure proved to contain the same human kappa-light chain as confirmed by sequence analysis. This human light chain furtheron called K8 was then combined with a human IgD-heavy chain library, that was again displayed on filamentous phage and selected in vitro by several rounds of panning on immobilized recombinant human 17-1A-antigen. Several Fab-fragments were expressed form several clones after each round of panning and again screened by ELISA for antigen binding. Sequence analysis of the binding entities enriched during the panning procedure revealed two different heavy chain-variable regions called D4.5 and D7.2 each of which combines with the K8-light chain to form different human antigen binding sites with specificity for the human 17-1A-antigen. The human light and heavy chain repertoires were cloned from several preparations of total RNA isolated from human blood and bone marrow samples of several donors by using kappa or lambda light chain specific as well as IgD-heavy chain specific RT-PCR. As it is impossible to selectively amplify the light chain repertoire that is combined with IgD-heavy chains in vivo, unless IgD-positive B-cells are purified for RNA-preparation, the light chain libraries used are not limited to the antibody repertoire of mature

unprimed or anergic B-lymphocytes. However, due to the predominance of the heavy chain in antigen recognition, this does not substantially undermine the advantages of the IgD-repertoire for selecting human antibodies to self-antigens. Further and most importantly due to the exposure to the human immune system selection of such light chains still guarantees a low immunogenic profile in humans.

In a further particularly preferred embodiment of the method of the invention, said surrogate chain is a mouse VH or VL chain.

In a further preferred embodiment of the method of the invention, said selection of a suitable combination involves

(a) testing one and the same VH chain in combination with a variety of different VL chains for binding to said human antigen; or

(b) testing one and the same VL chain in combination with a variety of different VH chains for binding to said human antigen.

This embodiment is advantageously employed again, if either the VL or the VH chains are known to specifically interact with the human target molecule. Then, an appropriate second chain can be selected on the basis of preferably an improved binding to the target molecule.

In a further preferred embodiment of the method of the invention, said method comprises the steps of obtaining, after selection, the human VH and VL chains or the corresponding nucleic acids and fusing said chains to the same or other VH or VL chains, to immunoglobulin constant regions of heavy (CH) or light chains (CL) or parts thereof or to other biologically active molecules such as peptides, proteins, nucleic acids, small organic compounds, hormones, neural transmitters, peptidomimics, PNAs (Milner, Nature Medicine 1 (1995), 879-880; Hupp et al., Cell 83 (1995), 237-245; Gibbs and Oliff, Cell 79 (1994), 193-198). The other functional molecule may be either physically linked by, e.g., chemical means to VH and VL chains or may be fused by recombinant DNA techniques well known in the art.

This embodiment of the invention is particularly useful for developing specific drugs that may be used to target desired antigens in the human body. For example, if tumor antigens are targeted, the VH and VL chains may, at the nucleic acid or amino acid level, be fused to a toxin moiety, thus resulting in an immunotoxin, to the extracellular portion of a cellular receptor or a soluble cytokine or parts thereof respectively, thus resulting in constructs enhancing the anti-tumor immune response or to an antibody-Fv-fragment thus resulting in a bispecific antibody derivative.

In a further particularly preferred embodiment of the method of the invention, said constant region chains are derived from human IgG1 or IgG3.

The constant region chains of human IgG1 or IgG3 are preferentially used if cells expressing the target antigen should be destroyed in the human body. It is well-known in the art that these IgG-subclasses efficiently mediate antibody dependent cellular cytotoxicity (ADCC) and contribute to the destruction of cells recognized and bound by these antibody subclasses.

In a further preferred embodiment of the method of the invention, said VH and/or VL chains are coupled with non-proteinous pharmaceuticals preferrably of low molecular weight such as radioisotopes or substances used for chemotherapy, thus resulting in a more specific in vivo targeting of said pharmaceuticals.

In a further preferred embodiment of the method of the invention, said VH or VL chains are expressed from nucleic acid sequences that are the result of the RT-PCR amplification of mRNA derived from essentially unprimed mature human B-lymphocytes or essentially anergic human B-cells.

It is preferred to amplify the VH or VL chains by RT-PCR once the suitable source thereof has been identified and isolated. It is preferred to use the mRNA of nucleated cells from human bone marrow or more preferable from human blood for amplifying VH or VL chains by RT-PCR as these two tissue compartments are the most easily accessible B-cell sources in humans. It is further preferred to isolate anergic B-cells or more preferable mature unprimed B-lymphocytes from the

nucleated cells of said tissue compartments by using e.g. magnetic beads or flow cytometry based cell sorting prior to RNA-preparation. This procedure guarantees that both, VH and VL chains amplified by RT-PCR, are derived from the preferred B-cells population. Alternatively, if mRNA of the whole fraction of nucleated cells from said tissue compartments is used to amplify VH or VL chains by RT-PCR, it is preferable to amplify the VH-region as half of the heavy chain Fd-segment (VH-CH1) of human IgD by using an IgD-specific 3' PCR-primer e.g. that enlisted in table 1 which exclusively gives raise to PCR-products from the human IgD-heavy chain, only expressed in mature unprimed human B-lymphocytes and in anergic human B-cells.

In a further preferred embodiment of the method of the invention, the anti-human receptor which is low or not immunogenic in humans, comprises a combination of functionally rearranged VH and VL chains preferably from essentially unprimed mature human B-lymphocytes or essentially anergic human B-cells and obtainable by the method according to any one of claims 1 to 17.

The advantages of the antibody of the invention have been outlined herein above. It has to be emphasized that corresponding antibodies directed against human antigens and derived from human sources, said antibodies having thus a low or no immunogenicity in humans, have so far not been isolated in the art. Accordingly, the antibodies of the invention are the starting point of a whole new development of antibodies that may be used in various fields of medicine and pharmacy.

In an additional preferred embodiment of the method of the invention, the receptor is an antibody or a fragment thereof.

In another preferred embodiment of the method of the invention, the receptor is specific for a human tumor antigen, most preferably for the human 17-1A antigen.

The invention furthermore relates to a receptor wherein said VH chain comprises one of the sequences shown in Fig. 6 (nucleotides 1 to 381) and Fig. 7 (nucleotides 1 to 339) and/or said VL chain comprises the sequence shown in Fig. 5 (nucleotides 1 to 321).

Furthermore, the invention relates to a VH chain or a part thereof comprised in the receptor of the invention.

The invention also relates to a VL chain or a part thereof comprised in the receptor of the invention.

In a further particularly preferred embodiment of the method of the invention, said part of said VH chain is the CDR3 domain.

Furthermore, the invention relates to a kit comprising a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least one of the VH and VL chains are derived from essentially unprimed mature human B lymphocytes or from essentially anergic human B cells, said chains being expressible from recombinant vectors of an in vitro display system.

Said kit is advantageously used in carrying out the method of the invention and thus obtaining receptors of desired specificity.

Preferably, in said kit, said in vitro display system is a phage display system.

The invention relates further to an antibody characterized in that it is derived from human sequences and is specific for the human 17-1A antigen.

With the method of the invention, for the first time a human antibody which is specific for the human 17-1A antigen has been developed. This development was no trivial task since human antibodies against antigens in the human body are usually removed as a consequence of the surveillance function of the immune system. Accordingly, for the first time now a human antibody has been developed that can advantageously be used in the monitoring and/or destruction of tumor cells carrying the 17-1A antigen.

In said antibody, said VH chain preferably comprises one of the two following sequences shown in Fig. 6 (nucleotides 1 to 381) and Fig. 7 (nucleotides 1 to 339) and/or said VL chain comprises the sequence shown in Fig. 5 (molecules 1 to 321).

This receptor and preferably antibody repertoire selected for low immunogenicity has been concluded to be best represented in a human IgD-antibody library. IgD is expressed as membrane antigen receptor together with surface IgM on mature unprimed B-lymphocytes that enter primary follicles during their traffic to and between secondary lymphoid organs unless they have encountered multivalent self antigen resulting in clonal deletion or soluble monovalent self antigen rendering them anergic and short lived due to exclusion form primary follicles. Except mature unprimed B-lymphocytes human IgD-libraries only represent the antibody repertoire of short-lived B-cells that have been rendered anergic in contact with soluble monovalent self antigen but are unlikely to contribute specific binding entities to human cell surface molecules resembling multivalent self-antigens that induce clonal deletion instead of B-cell anergy.

Moreover, the present invention relates to a pharmaceutical composition comprising at least one of the aforementioned receptors or parts thereof of the invention, either alone or in combination, and optionally a pharmaceutically acceptable carrier or excipient. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration.

Thus, the invention also relates to the use of a receptor or parts thereof produced according to the method of the invention for the preparation of a pharmaceutical composition for treating, preventing and/or delaying of a tumor, in a subject, preferably wherein the tumor is of epithelial origin.

The figures show:

Fig. 1: Cloning site of pComb3H with important restriction sites. The following abbreviations were used: P, promotor; VL, variable light chain domain; CL, constant light chain domain 1; VH, variable heavy chain domain; CH1, constant heavy chain domain 1; L1/2, procaryotic leader sequences.

Fig. 2 Scheme of the pComb3H-plasmid and the fully expressed M13-phage. On pComb3H the organization of leader (L) *ompA*, light chain, leader (L) *pelB*, heavy chain and *gene III* is shown. The fully expressed M13-phage displays on its surface the phenotype of a certain Fab-fragment consisting of a light chain and the Fd-segment of a heavy chain linked to the gene III product and contains the corresponding genotype as single-stranded DNA encoding the heavy and light chain of the displayed Fab-fragment

Fig. 3 ELISA of Fab fragments. Periplasma preparations of soluble Fab fragments each containing the Fd segment of chimerized M79 and a single human kappa chain per clone. Detection was carried out by a polyclonal-anti-human IgG/M/E antibody and measured at a wavelength of 405 nm (y-axis). Clones are presented on the x-axis, the first number indicates the round of panning, the second one is the clone number. Clones 1.5-9 have a combination of chimeric M79 Fd segment with one random kappa chain and represent negative controls.

Fig. 4 ELISA of Fab fragments. Periplasma preparations of soluble Fab fragments each containing the k8 light chain and a single human Ig delta chain Fd-segment. Detection was carried out by a polyclonal anti-human kappa antibody and measured at a wavelength of 405 nm (y-axis). Clones are presented on the x-axis, the first number indicates the round of panning, the second one is the clone number. Clones 1.2-6 have a combination of k8 light chain with one random Ig delta heavy chain Fd-segment and represent negative controls.

Fig. 5 DNA- and protein-sequence of the human kappa 8 variable region. Numbers indicate the nucleotide (nt) positions, amino acids are presented in single letter code. CDR1 includes nt 70 to nt 102, CDR2 nt 148 to nt 168, CDR3 nt 265 to nt 294.

Fig. 6 DNA-sequence of the human D4.5 variable region. Numbers indicate the nucleotide (nt) positions, amino acids are presented in the single letter code. CDR1 includes nt 91 to nt 105, CDR2 nt 148 to nt 198, CDR3 nt 292 to nt 351. The border between the heavy chain variable region and the CH1 domain of the Ig delta constant region is located between nt 382 and nt 383 with the delta constant region sequence starting at nt 384.

Fig. 7 DNA-sequence of the human D7.2 variable region. Numbers indicate the nucleotide (nt) positions, amino acids are presented in one letter code. CDR1 includes nt 91 to nt 105, CDR2 nt 148 to nt 198, CDR3 nt 292 to nt 309. The border between the heavy chain variable region and the CH1 domain of the Ig delta constant region is located between nt 340 and nt 341 with the delta constant region sequence starting at nt 343

Fig. 8 Flow cytometry on 17-1A positive Kato cells for testing binding activity of a periplasma preparation containing the k8-D4.5-Fab fragment. Kato-cells were incubated with I) irrelevant periplasma preparation, II) 10 µg/ml chimeric (bivalent !) M79, III) k8-D4.5 periplasma preparation and IV) 1:10 dilution of K8-D4.5 periplasma preparation. Relative cell number is shown on the y-axis, relative fluorescense intensity is shown on the x-axis.

Fig. 9 Cloning site of pEF-ADA with important restriction sites. The following abbreviations were used: P, promotor; VL, variable light chain domain; CL, constant light chain; Leuc, eucaryotic leader sequence.

Fig. 10 Cloning site of pEF-DHFR with important restriction sites. The following abbreviations were used: P, promotor; VH, variable heavy chain domain;

CH1/2/3, constant heavy chain domain 1/2/3; Leuc, eucaryotic leader sequence.

Fig. 11 SDS-PAGE of H79hu/M antibody preparations. Approx. 10 µg of each antibody were run on a 12.5 % denaturating Polyacrylamid-gel under reducing and non-reducing conditions and stained with coomassie blue.
Lane 1: Marker (MW [kDa] of single bands marked on the left side of the gel)
Lane 2: H79 humane IgG1 (non-reducing)
Lane 3: H79 humane IgG1 under reducing conditions
Lane 4: H79 murine IgG1 (non-reducing)
Lane 5: H79 murine IgG1 under reducing conditions

Fig. 12 Flow cytometry on 17-1A positive Kato cells for testing binding activity of purified H79 human IgG1 as well as purified H79 murine IgG1. Kato-cells were incubated with IgG controls (10 µg/ml human IgG1 and murine IgG1 respectively), M79 and chimerized M74 (both 17-1A specific), respectively, as positive controls (10 µg/ml), H79 human IgG1 and H79 murine IgG1 (10 µg/ml) as well as 1:10 dilutions of both. Relative cell number is shown on the y-axis, relative fluorescense intensity is shown on the x-axis.

Fig. 13 Light microscopic photo of stained healthy human colon tissue: positive control. 5nm cryosections of normal mucosa tissue were incubated with the murine M79 antibody as positive control (10µg/ml). Detection of bound murine antibodies was carried out with a peroxidase conjugated polyclonal anti-mouse-Ig antibody and stained with carbazole (brown-red). Counter staining was carried out with hemalaun (blue).

Fig. 14 Light microscopic photo of stained healthy human colon tissue: H79 murine IgG1. 5nm cryosections of normal mucosa tissue were incubated with the murine IgG version of the H79 antibody (10µg/ml). Detection of bound murine IgG1 antibodies was carried out with a peroxidase conjugated

polyclonal anti-mouse-Ig antibody stained with carbazole (brown-red). Counter staining was carried out with hemalaun (blue).

Fig. 15 Light microscopic photo of stained colon carcinoma: positive control. 5nm cryosections colon carcinoma were incubated with the murine antibody as positive control (10µg/ml). Detection of bound murine antibodies was carried out with a peroxidase conjugated polyclonal anti-mouse-Ig antibody and stained with carbazole (brown-red). Counter staining was carried out with hemalum (blue).

Fig. 16 Light microscopic photo of stained carcinoma: H79 murine IgG1. 5nm cryosections of colon carcinoma tissue were incubated with murine IgG1 version of the H79 antibody (10µg/ml). Detection of bound murine IgG1 antibodies was carried out with a peroxidase conjugated polyclonal anti-mouse-Ig antibody and stained with carbazole (brown-red). Counter staining was carried out with hemalaun (blue).

Fig. 17 Light microscopic photo of stained healthy human colon tissue and human colon carcinoma tissue: IgG2a isotype control. 5nm cryosections of normal mucosa and colon carcinoma tissue were incubated with irrelevante murine IgG2a antibody as negative control (10µg/ml). Detection of bound murine antibodies was carried out with a peroxidase conjugated polyclonal anti-mouse-Ig antibody and stained with carbazole (brown-red). Counter staining was carried out with hemalaun (blue).

Fig. 18 Light microscopic photo of stained healthy human colon tissue and colon carcinoma tissue: IgG1 isotype control. 5nm cryosections of normal mucosa and colon carcinoma tissue were incubated with irrelevante murine IgG1 antibody as negative control (10µg/ml). Detection of bound murine antibodies was carried out with a peroxidase conjugated polyclonal anti-mouse-Ig antibody and stained with carbazole (brown-red). Counter staining was carried out with hemalaun (blue).

The Examples illustrate the invention:

Exampl I: Construction of the combinat rial antibody library and phag display -

A library of human immunoglobulin (Ig) light chain and Ig heavy chain Fd-DNA-fragments was constructed by RT-PCR with kappa-, lambda- and Fd delta specific primer sets on the total RNA prepared from peripheral blood lymphocytes (PBL)- and bone marrow-samples of four and ten human donors, respectively according to Chomczynski, Analytical Biochemistry 162 (1987) 156-159. cDNA was synthesized according to standard methods (Sambrook, Cold Spring Harbor Laboratory Press 1989, second edition).

The following primer sets were chosen, giving rise to a 5'-*Spe*I and a 3'-*Xho*I recognition site for the heavy chain fragments and a 5'-*Sac*I and a 3'-*Xba*I recognition site for light chains:

For the PCR-amplification of the delta Fd cDNA-fragments five different 5'-VH-family specific primers were each combined with one 3'-CH1 delta primer; for the PCR-amplification of the kappa (K) light chain fragments five different 5'-VK-family specific primers were each combined with one 3'-CK primer and for the amplification of the lambda (L) light chain fragments, eight different 5'-VL-family specific primers were combined with one 3'-CL-primer. Primer sets for the amplification of the Fab DNA-fragments (5' to 3') are shown in Table I below.

The following PCR-programm was used for amplification:

Denaturation at 94°C for 15 seconds, primer annealing at 52°C for 50 seconds and primer extension at 72°C for 90 seconds for 40 cycles, followed by a 10 minutes final extension at 72°C.

Table I

heavy chain Fd-fragment:	
5'-primer:	
VH1,3,5,7:	AGGTGCAGCTGCTCGAGTCTGG
VH2:	CAG(A/G)TCACCTTGCTCGAGTCTGG
VH4:	CAGGTGCAGCTGCTCGAGTCGGG
VH4B:	CAGGTGCAGCTACTCGAGTGGGG
VH6:	CAGGTACAGCTGCTCGAGTCAGG
3'-primer:	
CD1:	TGCCTTACTAGTCTCTGGCCAGCGGAAGAT
kappa chain fragment:	
5'-primer:	
VK1:	GAGCCGCACGAGCCCGAGCTCCAGATGACCCAGTCTCC
VK3:	GAGCCGCACGAGCCCGAGCTCGTG(A/T)TGAC(A/G)CAGTCTCC
VK2/4:	GAGCCGCACGAGCCCGAGCTCGTGATGAC(C/T)CAGTCTCC
VK5:	GAGCCGCACGAGCCCGAGCTCACACTCACGCAGTCTCC
VK6:	GAGCCGCACGAGCCCGAGCTCGTGCTGACTCAGTCTCC
3'-primer:	
CK1D:	GCGCCGTCTAGAATTAACACTCTCCCCTGTTGAAGCTCTTTGTGA CGGGCGAACTCAG

450 ng of the kappa light chain fragments were ligated with 1400 ng of the phagmid pComb3H derived from pComb3 (Barbas, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 7978-7982) wherein the heavy chain position was already occupied by the Fd

fragment of the chimerized murine antibody M79 directed against the extracellular part of the 17-1A protein (see Fig. 1 for pComb3H cloning site).

The resulting combinatorial antibody library was then transformed into 300 µl of electrocompetent *Escherichia coli* XL1 Blue by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 FD, 200 Ohm, Biorad gene-pulser) reaching a library size of 4×10^7 independent clones. After one hour of phenotype expression, positive transformants were selected for carbenicilline resistance encoded by the pComb vector. After this adaption these clones were infected with an infection dose of 1×10^{12} phage particles of the helper phage VCSM13 resulting in the production and secretion of filamentous M13 phages, each of them containing single stranded pComb3H-DNA encoding a single human light chain and the Fd segment of chimeric M79 and displaying the corresponding Fab fragment on the phage surface as a translational fusion to phage coat protein III (phage display); see Fig. 2.
This phage library carrying the cloned Fab repertoire was harvested from the culture supernatant by PEG8000/NaCl precipitation and centrifugation, redissolved in TBS/1%BSA and incubated with recombinant s17-1A immobilized on 96 well ELISA plates. s17-1A was prepared as described (Mack, Proc. Natl. Sci. U.S.A. 92 (1995) 7021-7025). Fab phages that did not specifically bind to the target antigen were eliminated by up to ten washing steps with TBS/Tween. Binding entities were eluted by using HCl-Glycine pH 2.2 and after neutralization of the eluat with 2 M Tris pH 12, used for infection of a new uninfected *E. coli* XL1 Blue culture. Cells successfully transduced with a pComb phagmid copy, encoding an antigen binding Fab fragment, were again selected for carbenicilline resistance and subsequently infected with VCMS13 helper phage to start the second round of antibody display and in vitro selection.
After five rounds of production and selection of antigen-binding Fab phages, plasmid DNA containing the selected Fab repertoire was prepared.

For the production of soluble Fab proteins the *gene III SpeI-NheI* DNA fragment was excised from the plasmids and thus destroying the translational fusion of the Fd heavy chain segment with the *gene III* protein. After religation this pool of plasmid DNA was transformed into 100 µl heat shock competent *E. coli* XL1 Blue and plated

on Carbenicilline (Carb) LB- Agar. Single colonies were grown in 10 ml LB-Carb-cultures/20 mM $MgCl_2$ and Fab expression was induced after six hours by adding Isopropyl-β-D-thiogalactosid (IPTG) to a final concentration of 1 mM. This in vitro selection procedure as well as expression of soluble Fab-fragments was carried out according to Burton, Proc. Natl. Acad. Sci. U.S.A. 88 (1991), 10134-10137. The cells were harvested after 20 hours; periplasma preparation was carried out by osmotic shock and tested by ELISA according to standard methods for Fab fragments binding to s17-1A. 23 out of 27 clones showed binding activity. After sequenzing the two clones with the strongest signals (see Fig. 3) turned out to have identical kappa chains and were called k8; see Fig. 5.

This human kappa light chain k8 was now used as a binding partner for the Ig delta heavy chain pool; 2250 ng of human delta heavy chain Fd DNA-fragments were ligated with 7000 ng of the phagmid vector pComb3H containing the k8 DNA-fragment in the light chain position.
The choice of the human delta chain repertoire as source for heavy variable-regions that specifically bind to the 17-1A antigen when combined with the k8 light chain, appeared to be most suitable. Delta chains are only produced in mature unprimed and in self-antigen specific anergic B-cells; therefore their diversity is higher and the number of each single specifity lower than those of other heavy chains.

The transformation of the pComb-k8-delta library into 1500 µl *E. coli* XL1 Blue by five electroporations (2.5 kV, 0.2 cm gap cuvette, 25 FD, 200 Ohm) resulted in a total number of 1.1×10^9 independent clones.
In vitro selection of this combinatorial antibody library was carried out as described above for the human light chain repertoire. After four rounds of panning soluble Fab fragments were prepared from eight clones.
The periplasma preparations were tested on ELISA. One of the clones showed strong antigen binding (see Fig. 4). This clone was called D4.5 and the DNA of the Fd delta fragment was sequenzed with a reverse delta CH1-specific primer (see Fig. 6).
Another s17-1A binding Fab fragment was isolated after further rounds of panning first appearing in round seven with a markedly weaker ELISA signal compared to

D4.5 (see Fig. 4). The clone was designated as D7.2 and the DNA sequence was determined using again the delta specific primer (see Fig. 7).

Example II: Bacterial expression in *E. coli* XL1 Blue

As previously mentioned, *E. coli* XL1 Blue transformed with pComb3H containing a light and the Fd-segment of a heavy chain produce soluble Fab in sufficient amounts after excision of the gene III fragment and induction with 1 mM IPTG. The heavy chain Fd-segment and the light chain are exported into the periplasma where they assemble and form functional Fab.

For better periplasma preparations the cells were grown in SB-medium supplemented with 20 mM $MgCl_2$ and are redissolved in PBS after harvesting. By four rounds of freezing at -70°C and thawing at 37°C, the outer membrane of the bacteria was destroyed by osmotic shock and the soluble periplasmatic proteins including the Fab fragments were released into the supernatant. After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the k8-D4.5-Fab-antibody-fragment was collected and used for further examination.

First the k8-D4.5-Fab was tested for binding to immobilized s17-1A antigen and showed a strong ELISA signal (see example I).

Detection of k8-D4.5 Fab bound to immobilized s17-1A anigen was carried out using a polyclonal biotinylated anti-human-kappa antibody (1µg/ml PBS) detected with horse raddish conjugated Avidine (1µg/ml PBS). The signal was developed by adding a substrate solution, containing 2,2'Azino-bis(3-Ethylbenz-Thiazoline-6-Sulfonic Acid) and Na-perborate and detected at a wavelength of 405 nm.

The test for binding on 17-1A expressing Kato-cells was also carried out with the periplasma preparation.

200 000 Kato cells were successively incubated with periplasma preparation containing the k8-D4.5-Fab, biotinylated polyclonale anti-human-kappa antibody (20 µg/ml PBS) and FITC-conjugated Streptavidine and subsequently analyzed by flow cytometry.

The periplasma preparation containing the k8-D4.5-Fab showed a distinct signal compared to the irrelevant periplasma preparation (negative control) indicating specific binding of this human Fab fragment to 17-1A positive cells (see Fig. 8).

Example III: Eucaryotic expression in CHO-cells

Bacteria are usually not capable of producing complete functional immunoglobulins although they express functional Fab fragments.
For the production of functional antibodies mammalian cells have to be used and therefore the k8-light chain and the variable domain of D4.5 heavy chain were subcloned in mammalian expression vectors.

a.) light chain k8: To generate suitable terminal restriction sites, the k8 DNA fragment was reamplified by PCR resulting in a k8 fragment with a *Bsu*36I-site at the 5'-end as well as a *Sal* I and a *Not* I-site at the 3'-end.
This fragment was subcloned into the plasmid BSPOLL by *Bsu*36I and *Not* I, thus adding a mammalian leader sequence and sequenzed for preventing PCR-induced mutations.
Utilizing EcoRI and Sal I, k8 was excised and cloned into the eucaryotic expression vector pEF-ADA (see Fig. 9) derived from the expression vector pEF-DHFR (Mack, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7021-7025) by replacing the cDNA encoding murine dihydrofolate reductase (DHFR) by that encoding murine adenosine deaminase (ADA).

10^7 CHO cells were transfected with 100 µg of linearized plasmid DNA and then cultured under culture conditions selecting for adenosine desaminase (ADA) activity encoded by the expression vector.
Surviving ADA-positive cells were cultured for further transfection with the heavy chain and designated pEFADAH79k8.

b.) heavy 4.5 variable domain: From the delta Fd-fragment D4.5, the variable region was reamplified by PCR generating *Bsu*36I restriction sites at both ends.

The V-D4.5 DNA-fragment was then subcloned with these restriction sites into the eucaryotic expression vector pEF-DHFR between an eucaryotic leader and a human IgG1 constant region (see Fig. 10).

The variable region was sequenced and the complete clone was called H79V-D4.5 hu IgG1.

For later tissue staining, the human IgG1 constant region was replaced by the murine IgG1 constant region using *Xba* I for subcloning. This plasmid was designated H79V-D4.5 MIgG1.

Both, the human and the murine IgG1-version of D4.5 were each transfected into 10^7 CHO-cells, already expressing the k8 light chain, by using 100 µg linearized plasmid DNA respectively.

Positive cells were grown under conditions selecting for ADA- and dihydrofolate reductase (DHFR) - activity resulting in the cell lines H79-huIgG1 and H79-MIgG1. Selection for ADA-and DHFR-activity was carried out as described (Kaufman, Methods Enzymol. 185 (1990), 537-566).

Three days old culture supernatant of a confluent 30 ml culture was tested for binding to immobilized s17-1A by ELISA and revealed an approximate concentration of 0.2 µg H79 antibody and an estimated binding affinity in the range of the murine antibody M79 for both, the murine and the human IgG-version.

Large scale antibody production was carried out in rollerbottles using 500 ml medium.

The H79-huIgG1 antibody was purified by using a protein A affinity column.

The H79-MIgG1 antibody was purified by anti-mouse IgG affinity chromatography.

Purity and molecular weight of the recombinant antibodies were determined by SDS-PAGE under reducing and nonreducing conditions (see Fig. 11).

Protein purification and SDS-PAGE were carried out according to standard procedures.

Example IV: Functional analysis of the H79 antibodies

IV.1. Test on immobilized antigen

Three days old culture supernatant of a confluent 30 ml culture of human and murine IgG1-transfectants respectively as well as the corresponding preparations of purified antibody were tested for binding on immobilized s17-1A antigen by ELISA and compared to the murine M79 anti 17-1A antibody.
Detection was carried out as described in II.
Both H79 antibodies revealed identical binding affinities in the range of the murine M79.

IV.2. Flow cytometry on 17-1A expressing eucaryotic cells

Both, the purified human and the murine IgG H79 antibody were tested by FACS analysis on 17-1A expressing Kato cells (10 µg/ml antibody each).
2 x 10^5 cells were incubated with H79-huIgG1- or H79-MIgG1-supernatant, respectively. Detection of cell-bound H79 was carried out with FITC labeled anti mouse IgG- or anti human IgG antibodies, respectively.
Both antibodies showed distinct binding on the cells compared to the IgG negative-controls (see Fig. 12).

IV.3. Test on human tissue

5 nm cryosections of a colon carcinoma and normal colon tissue respectively were incubated with the murine IgG H79 antibody (10 µg/ml). In this experiment the murine IgG1 version of H79 was used to avoid unspecific staining due to the presence of human antibodies in human tissue. Detection of bound H79M was carried out with peroxidase conjugated polyclonal anti mouse Ig antibodies and stained with carbazole. Counter staining was carried out with hemalaun.
Results were evaluated by light microscopy.
H79 as well as the murine M79 positive control showed strong staining on healthy colon mucosa (M79, Fig. 13; H79, Fig. 14) and lighter staining on colon carcinoma

cells (M79, Fig. 15; H79, Fig. 16). In contrast, Isotype controls showed no staining on colon mucosa and colon carcinoma tissues (for M79; Fig. 17 and for H79, Fig. 18).

CLAIMS

1. A method for the production of an anti-human antigen receptor that is low or not immunogenic in humans comprising the steps of selecting a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least said VH chain is derived from essentially unprimed mature human B-lymphocytes or from essentially anergic human B cells and said VL chain is derived from a naturally occurring human B cell repertoire, said chains being expressed from a recombinant vector and using an in vitro display system for binding to a human antigen.

2. The method according to claim 1 wherein said receptor is an immunoglobulin or a fragment thereof.

3. The method according to claim 2 wherein said immunoglobulin fragment is a Fv-fragment.

4. The method according to any one of claims 1 to 3 wherein at least said VH and optionally said VL immunoglobulin chains are derived from a human IgD repertoire.

5. The method according to any one of claims 1 to 4 wherein said in vitro display system is a phage display system.

6. The method according to any one of claims 1 to 5 wherein said combination of rearranged chains is expressed from one or more different libraries.

7. The method according to any one of claims 1 to 6 wherein said human antigen is a tumor antigen.

8. The method according to claim 7 wherein said tumor antigen is the human 17-1A antigen.

9. The method according to claim 8 wherein said VH chain comprises one of the two sequences shown in Fig. 6 (nucleotides 1 to 381) and Fig. 7 (nucleotides 1 to 339) and/or said VL chain comprises the sequence shown in Fig. 5 (nucleotides 1 to 321).

10. The method according to any one of claims 1 to 9 wherein said selection step involves

(i) binding of the display vehicle expressing an antigen receptor

(a) on immobilized target antigen or fragments thereof;

(b) on optionally labeled cells expressing the target antigen or fragments thereof;

(c) or to soluble, preferably labeled target antigen or fragments thereof;

(ii) washing off non-specifically binding display vehicle (a and b) and subsequent elution of specifically binding display vehicle

or

(iii) positive enrichment of target antigen bound display vehicle (b and c) from target antigen solution or from suspensions of cells expressing the target antigen;

thus isolated display vehicles including their antigen receptors optionally being multiplied by replication and subjected to further rounds of in vitro selection as described in (i) to (iii).

11. The method according to any one of claims 1 to 10 wherein prior to said selection step either said VH or said VL chain is selected for binding to said antigen together with a surrogate V chain.

12. The method according to claim 11 wherein said surrogate chain is a mouse VH or VL chain.

13. The method according to any one of claims 1 to 12 wherein said selection of a suitable combination involves

(a) testing one and the same VH chain in combination with a variety of different VL chains for binding to said human antigen; or

(b) testing one and the same VL chain in combination with a variety of different VH chains for binding to said human antigen.

14. The method according to any one of claims 1 to 13 further comprising the steps of obtaining, after selection, the human VH and VL chains or the corresponding nucleic acids and fusing said chains to the same or other VH or VL chains, to immunoglobulin constant regions of heavy (CH) or light chains (CL) or parts thereof or to non-immunoglobulin chains and the corresponding nucleic acids, respectively.

15. The method according to claim 14 wherein said constant region chains are derived from human IgG1 or IgG3.

16. The method according to any one of claims 1 to 13 further comprising the steps of obtaining, after selection, the human VH and VL chains and physically linking said chains to non-proteinous pharmaceuticals and/or other biologically active molecules.

17. The method according to any one of claims 1 to 16 wherein said VH or VL chains are expressed from nucleic acid sequences that are the result of the RT-PCR amplification of mRNA derived from essentially unprimed mature human B-lymphocytes or from essentially anergic human B-cells.

18. An anti-human antigen receptor that is low or not immunogenic in humans, comprises a combination of functionally rearranged VH and VL chains preferably from essentially unprimed mature human B-lymphocytes or from essentially anergic human B-cells and obtainable by the method according to any one of claims 1 to 17.

19. The receptor according to claims 18 which is an antibody or a fragment thereof.

20. The receptor according to claim 18 or 19 which is specific for a human tumor antigen.

21. The receptor according to claim 20 which is specific for the human 17-1A antigen.

22. The receptor according to claim 21 wherein said VH chain comprises one of the following two sequences shown in Fig. 6 (nucleotides 1 to 381) and Fig. 7 (nucleotides 1 to 339) and/or said VL chain comprises the sequence shown in Fig. 5 (nucleotides 1 to 321).

23. A VH chain or a part thereof comprised in the receptor of any one of claims 18 to 22.

24. A VL chain or a part thereof comprised in the receptor of any one of claims 18 to 22.

25. The chain of claim 23 or 24 wherein said part is the CDR3 domain.

26. A kit comprising a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least one of the VH and VL chains are derived from essentially unprimed mature human B-lymphocytes or from essentially anergic human B-cells, said chains being expressible from recombinant vectors of an in vitro display system.

27. The kit according to claim 26 wherein said in vitro display system is a phage display system.

28. An antibody characterized in that it is derived from human sequences and is specific for the human 17-1A antigen.

29. The antibody according to claim 28, wherein the VH chain comprises one of the following two sequences shown in Fig. 6 (nucleotides 1 to 381) and Fig. 7 (nucleotides 1 to 339) and the VL chain comprises the sequence shown in Fig. 5 (nucleotides 1 to 321).

30. A pharmaceutical composition comprising a receptor of any one of claims 18 to 22, a VH chain of claim 23 or 25, a VL chain of claim 24 or 25 and/or antibody of claim 28 or 29, and optionally a pharmaceutically acceptable carrier.

Abstract

Described is a method for the production of an anti-human antigen receptor that is low or not immunogenic in humans comprising the steps of selecting a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least said VH chain is derived from essentially unprimed mature human B-lymphocytes or from essentially anergic human B cells and said VL chain is derived from a naturally occurring human B cell repertoire, said chains being expressed from a recombinant vector and using an in vitro display system for binding to a human antigen. Furthermore, receptors that are low or not immunogenic in humans and directed to human antigens are provided, said receptors being obtainable by the method of th invention. Said receptors are preferably antibodies or fragments thereof or immunoconjugates comprising the VH/VL chains of said antibody. In particular, receptors are described directed to human tumor antigens, preferably to the human tumor antigen 17-1A, also known as EpCAM, EGP or GA 733-2. Finally, kits useful for carrying out the method of the invention and pharmaceutical compositions comprising the aforementioned receptors are provided.

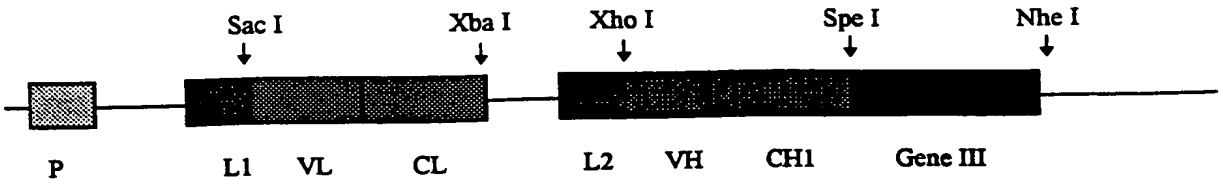


Fig. 1

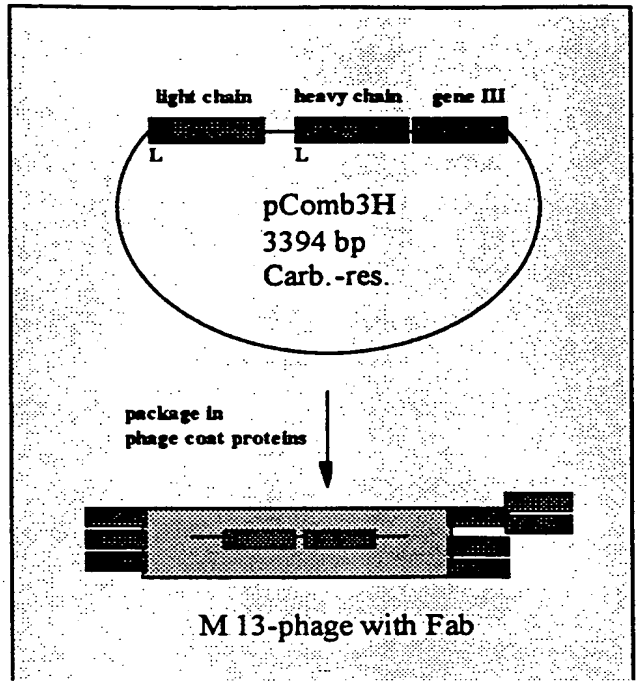

light chain
heavy chain
gene III
L
L
pComb3H
3394 bp
Carb.-res.
package in
phage coat proteins
M 13-phage with Fab

Fig. 2

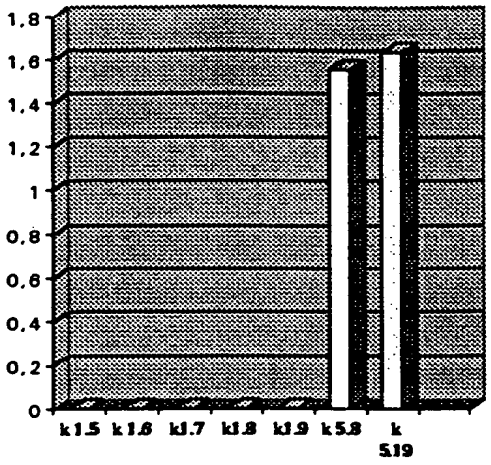


Fig. 3

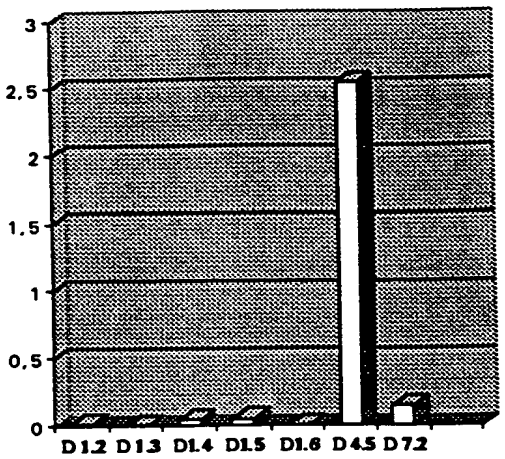


Fig. 4

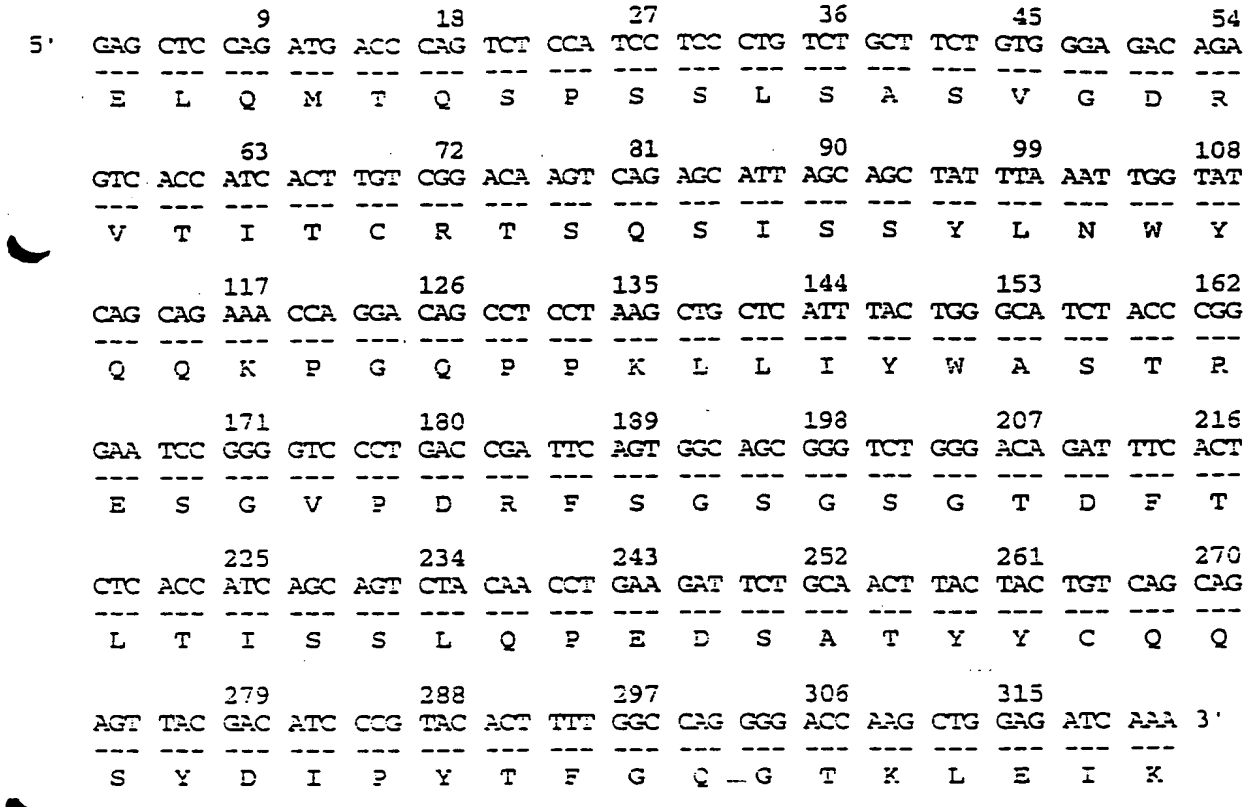

5' GAG CTC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCT TCT GTG GGA GAC AGA
E L Q M T Q S P S S L S A S V G D R
GTC ACC ATC ACT TGT CGG ACA AGT CAG AGC ATT AGC AGC TAT TTA AAT TGG TAT
V T I T C R T S Q S I S S Y L N W Y
CAG CAG AAA CCA GGA CAG CCT CCT AAG CTG CTC ATT TAC TGG GCA TCT ACC CGG
Q Q K P G Q P P K L L I Y W A S T R
GAA TCC GGG GTC CCT GAC CGA TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT
E S G V P D R F S G S G S G T D F T
CTC ACC ATC AGC AGT CTA CAA CCT GAA GAT TCT GCA ACT TAC TAC TGT CAG CAG
L T I S S L Q P E D S A T Y Y C Q Q
AGT TAC GAC ATC CCG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG ATC AAA 3'
S Y D I P Y T F G Q G T K L E I K

Fig. 5

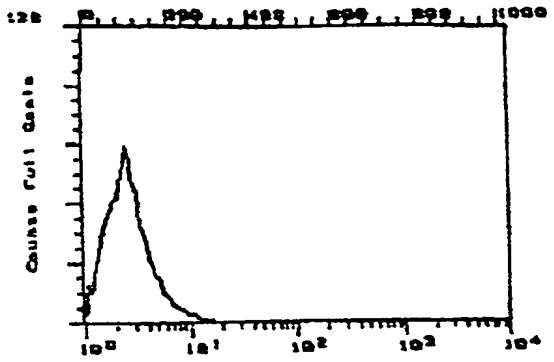
```
                    9                   18                  27                  36                  45                  54
5'  GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     E   V   Q   L   L   E   S   G   G   G   V   V   Q   P   G   R   S   L

                   63                  72                  81                  90                  99                 108
    AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT GGC ATG CAC TGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   L   S   C   A   A   S   G   F   T   F   S   S   Y   G   M   H   W

                  117                 126                 135                 144                 153                 162
    GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   S   Y   D

                  171                 180                 189                 198                 207                 216
    GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R

                  225                 234                 243                 252                 261                 270
    GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D

                  279                 288                 297                 306                 315                 324
    ACG GCT GTG TAT TAC TGT GCG AAA GAT ATG GGG TGG GGC AGT GGC TGG AGA CCC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     T   A   V   Y   Y   C   A   K   D   M   G   W   G   S   G   W   R   P

                  333                 342                 351                 360                 369                 378
    TAC TAC TAC TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     Y   Y   Y   Y   G   M   D   V   W   G   Q   G   T   T   V   T   V   S

                  387                 396                 405                 414
    TCA GCA CCC ACC AAG GCT CCG GAT GTG TTC CCT CTA 3'
    --- --- --- --- --- --- --- --- --- --- --- ---
     S   A   P   T   K   A   P   D   V   F   P   L
```

Fig. 6

```
                9                  18                  27                  36                  45                  54
5' GAG GTG CAG CTG CTC GAG TCT GGG GGA GTC GTG GTA CAG CCT GGG GGG TCC CTG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    E   V   Q   L   L   E   S   G   G   V   V   V   Q   P   G   G   S   L

               63                  72                  81                  90                  99                 108
   AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT GAT GAT TAT GCC ATG CAC TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    R   L   S   C   A   A   S   G   F   T   F   D   D   Y   A   M   H   W

               117                 126                 135                 144                 153                 162
   GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   S   Y   D

               171                 180                 189                 198                 207                 216
   GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R

               225                 234                 243                 252                 261                 270
   GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D

               279                 288                 297                 306                 315                 324
   ACG GCT GTG TAT TAC TGT GCG AAA AAG GAA GGC TAC TGG GGC CAG GGA ACC CTG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    T   A   V   Y   Y   C   A   K   K   E   G   Y   W   G   Q   G   T   L

               333                 342                 351                 360                 369
   GTC ACC GTC TCC TCA GCA CCC ACC AAG GCT CCG GAT GTG TTC CCT CTA 3'
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    V   T   V   S   S   A   P   T   K   A   P   D   V   F   P   L
```

Fig. 7

I)



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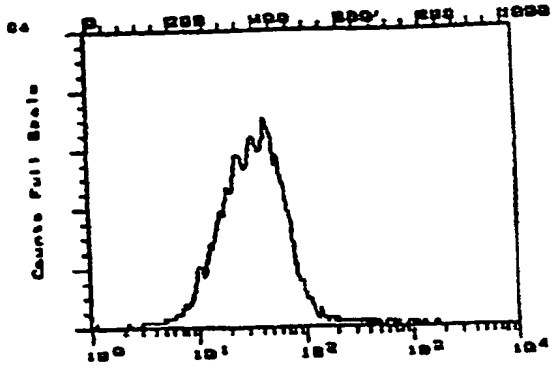
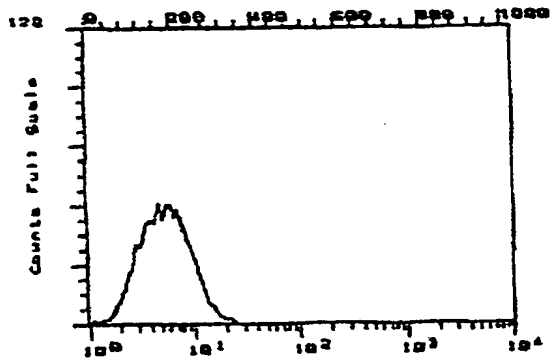


III)



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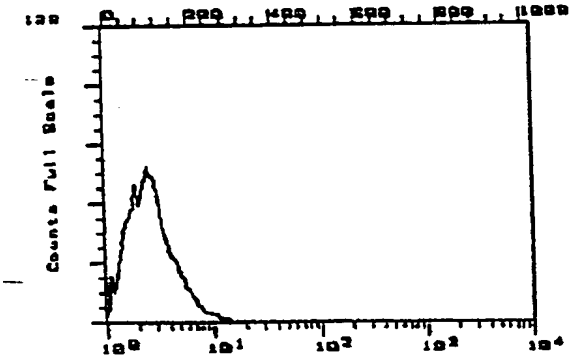


Fig. 8

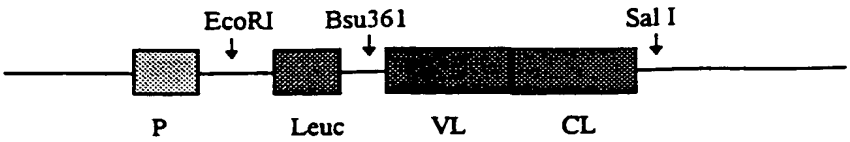


Fig. 9

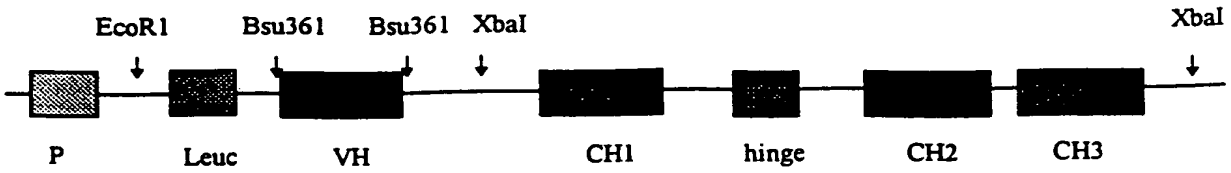


Fig. 10

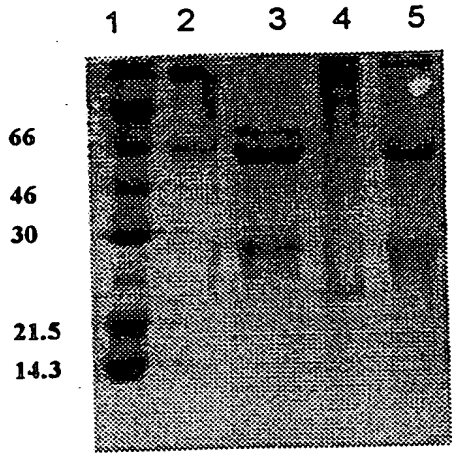
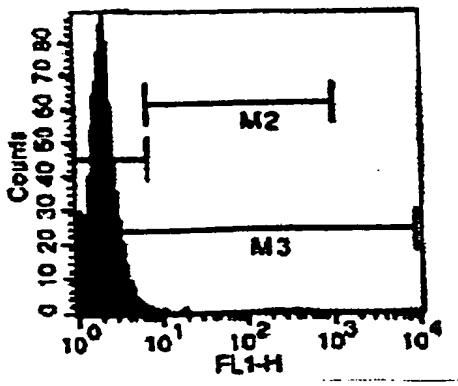
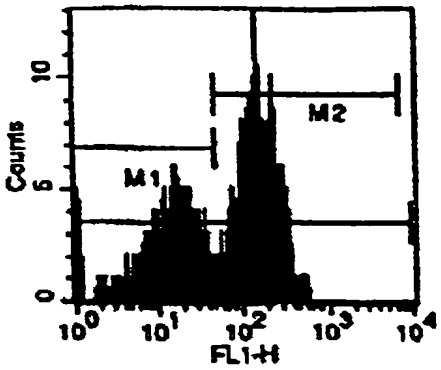


Fig. 11

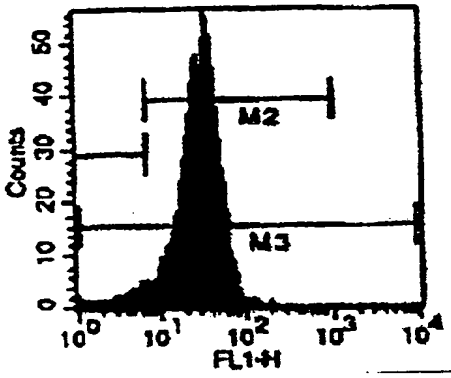
Human IgG:



M74ch:



H79huIgG:



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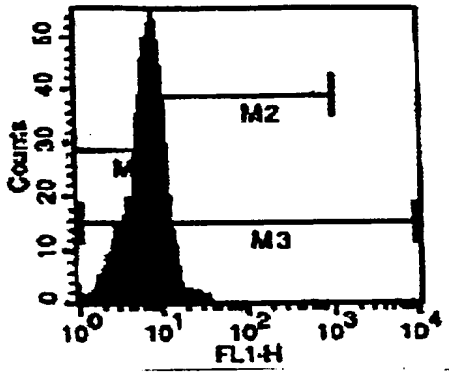
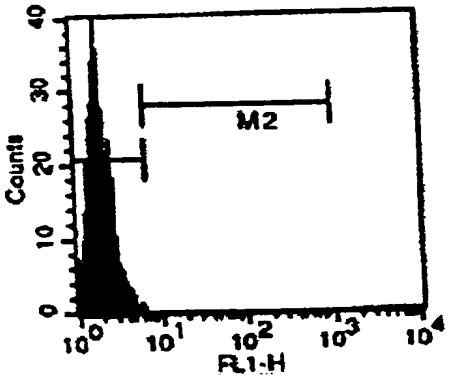


Maus IgG :



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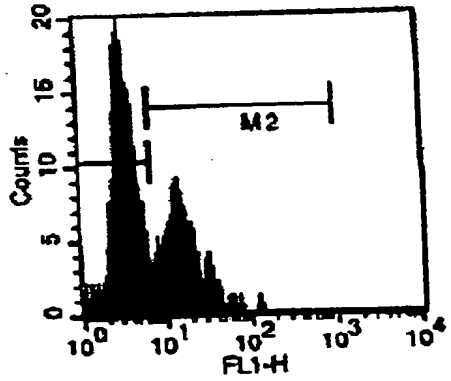

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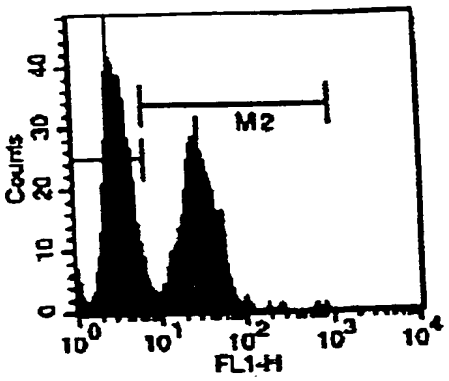

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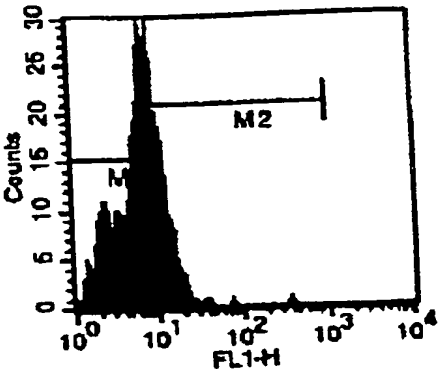

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

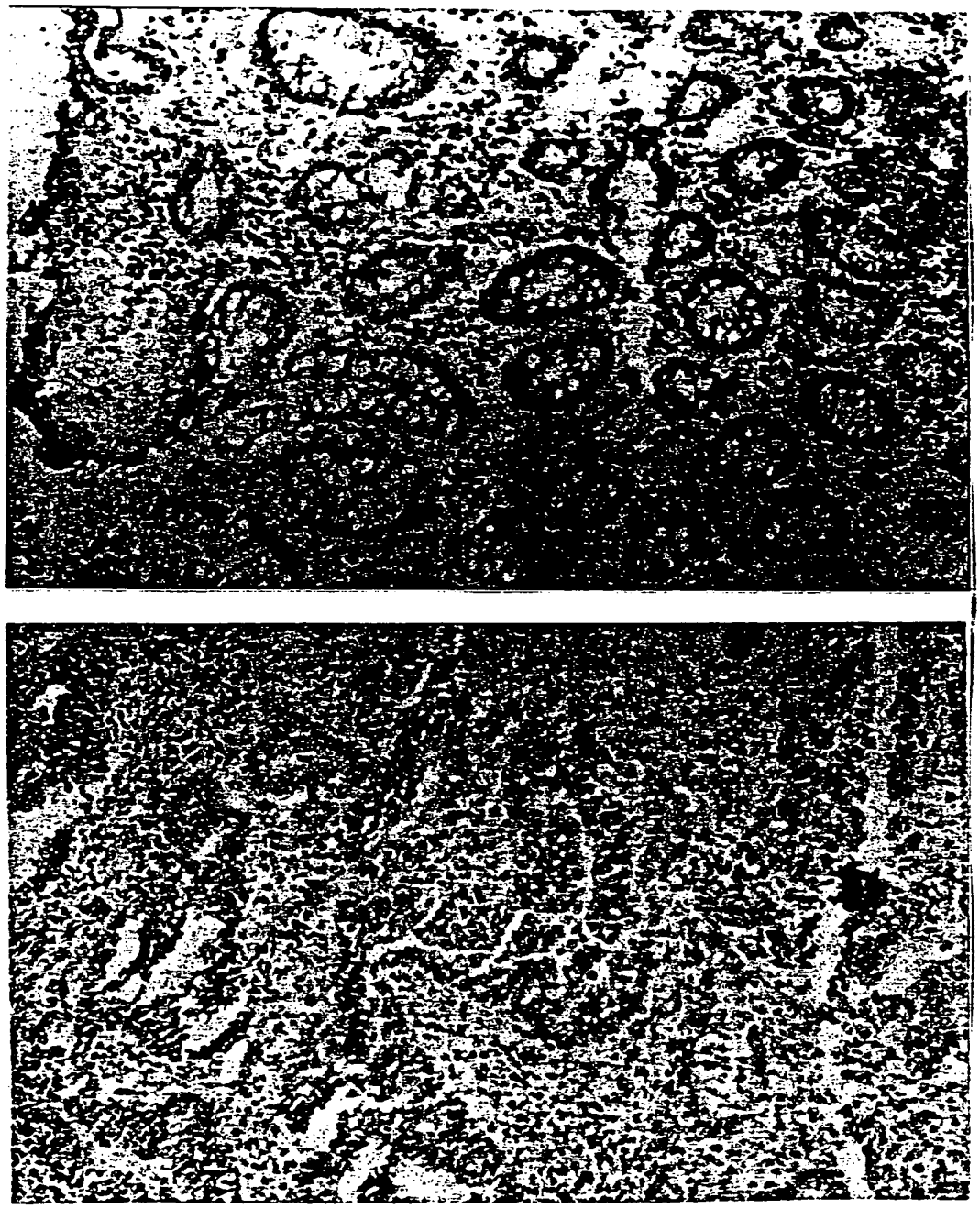

Fig. 17

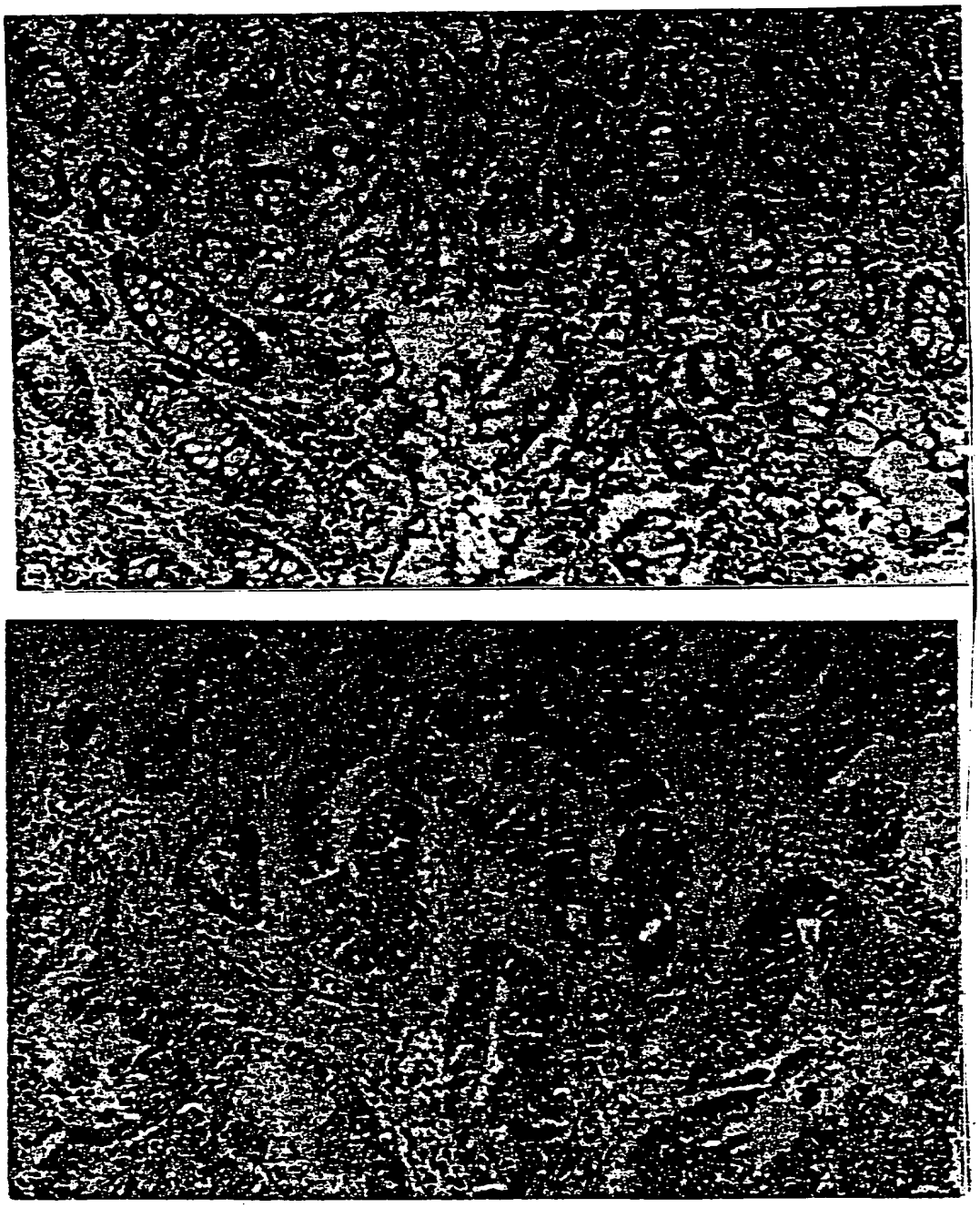

Fig. 18